

Spray-Dried Anti-*Campylobacter* Bacteriophage CP30A Powder Suitable for Global Distribution without Cold Chain Infrastructure

Nicholas B. Carrigy^a, Lu Liang^b, Hui Wang^a, Samuel Kariuki^c, Tobi E. Nagel^d, Ian F.

Connerton^b, Reinhard Vehring^{a*}

5 ^a Department of Mechanical Engineering, University of Alberta, Edmonton, Canada

^b School of Biosciences, University of Nottingham, Loughborough, UK

^c Centre for Microbiology Research, Kenyan Medical Research Institute, Nairobi, Kenya

^d Phages for Global Health, Oakland, USA

* Corresponding Author

10 Prof. Reinhard Vehring

University of Alberta, Department of Mechanical Engineering

10-203 Donadeo Innovation Centre for Engineering

9211 116th Street NW, Edmonton, Alberta, T6G 1H9, Canada

Telephone: +1 780 492 5180

15 E-mail: reinhard.vehring@ualberta.ca

Abstract

Campylobacter jejuni is a leading cause of foodborne illness globally. In this study, a spray drying and packaging process was developed to produce a thermally-stable dry powder containing bacteriophages that retains biological activity against *C. jejuni* after long distance
20 shipping at ambient temperature. Spray drying using a twin-fluid atomizer resulted in significantly less ($p < 0.05$) titer reduction than spray drying using a vibrating mesh

nebulizer. The use of centrifugation and dilution of filtered bacteriophage lysate in the formulation step resulted in a significantly greater ($p < 0.05$) proportion of bacteriophages remaining active relative to use of no centrifugation and dilution. The spray-dried

bacteriophage powder generated using leucine and trehalose as excipients was flowable, non-cohesive, and exhibited a high manufacturing yield. The powder retained its titer with no significant differences ($p > 0.05$) in biological activity after storage in suitable packaging for at least 3 weeks at room temperature and after ambient temperature shipping a total distance of approximately 19,800 kilometers, including with a 38°C temperature excursion.

The bacteriophage powder therefore appears suitable for global distribution without the need for cold chain infrastructure.

Keywords: *Campylobacter jejuni*, global health, particle engineering, phage CP20, phage CP30A, shipping stability

Abbreviations: CFU = colony-forming unit; PFU = plaque-forming unit; phage =

bacteriophage; SEM = scanning electron micrograph; TEM = transmission electron micrograph

1 Introduction

The gram-negative bacterium *Campylobacter jejuni* causes foodborne illness globally (Kaakoush et al., 2015). *C. jejuni* is prevalent in the gut of chickens and can be present and persist at high concentrations in raw or improperly-cooked meat with an attendant high risk of cross-contamination (Osano & Arimi, 1999; Kittler et al., 2013; Wagenaar et al., 2013; Kaakoush et al., 2015). In Kenya, *C. jejuni* is one of many foodborne bacteria that poses a substantial threat of mortality to children (O'Reilly et al., 2012; World Health Organization,

2012; Carron et al., 2018). Unfortunately, antibiotic-resistant *C. jejuni* strains are becoming
45 increasingly prevalent (Moore et al., 2006; Kaakoush et al., 2015).

Bacteriophages (phages) are viruses ubiquitous in the global environment, their presence
dependent on the availability of suitable bacterial hosts. Phages active against *C. jejuni*,
including antibiotic-resistant strains, have been isolated from chickens and may be useful
for decontamination and biocontrol of *C. jejuni* (Loc Carrillo et al., 2005; Wagenaar et al.,
50 2005; Kittler et al., 2013; Janež et al., 2014; Firlieyanti et al., 2016). An advantage of using
phages instead of broad spectrum antibiotics is that one species of phage typically infects
only a narrow spectrum of bacteria, thus not substantially impacting other beneficial
community members of the microbiota (Kutter and Sulakvelidze, 2005; Kutter et al., 2010;
Loc-Carrillo et al., 2011; Richards et al., 2019). Also, phages do not interact directly with
55 animal or human cells, thereby minimizing any unwanted side effects.

Spray drying can be used to produce dry powder that has improved thermal stability relative
to liquid dosage forms, potentially removing the need for cold chain shipping (Carrigy &
Vehring, 2019). Additionally, dry powder has less weight and volume compared to liquid,
which can reduce transportation costs and storage space requirements (Walters et al.,
60 2014). Compared to another commonly used method for powder production, lyophilization,
spray drying is a low-cost process (Quinn, 1965; Roser, 1991; Desai et al., 2005; Holsinger et
al., 2010; Schwartzbach, 2011; Anandharamakrishnan and Ishwarya, 2015; Siew, 2016;
Huang et al., 2017). Additionally, lyophilization is a lengthy batch process that is difficult to
scale-up and typically requires subsequent milling or sieving to adjust the particle size (Hoe
65 et al., 2014; Ledet et al., 2015), whereas spray drying is a fast, continuous, and scalable
process for producing powder with controlled particle size, distribution, and density, as well
as surface composition, roughness, and flowability (Vehring, 2008).

Leucine and trehalose are relatively low-cost excipients that have been used successfully for spray drying phages. Matinkhoo et al. (2011) dried *Burkholderia* phages in a leucine and trehalose formulation using a Büchi B-90 spray dryer and found $\sim 0.8 \log_{10}(\text{PFU/mL})$ titer reduction for *Myoviridae* phage KS4-M and $\sim 0.4 \log_{10}(\text{PFU/mL})$ titer reduction for *Myoviridae* phage KS14. Vandenheuvel et al. (2013) dried *Podoviridae* phage LUZ19 active against *Pseudomonas* and *Myoviridae* phage Romulus active against *Staphylococcus* using a ProCepT Micro spray dryer and found $\sim 0.02 \log(\text{PFU/mL})$ titer reduction for phage LUZ19 and $\sim 2.6 \log(\text{PFU/mL})$ for phage Romulus when spray dried using trehalose, which outperformed the use of either lactose or dextran 35 for stabilizing the phages. It was found that on storage these phages were susceptible to inactivation if the trehalose crystallized (Vandenheuvel et al., 2014). Leung et al. (2017) observed $\sim 1.3 \log_{10}(\text{PFU/mL})$ titer reduction due to formulation and spray drying of *Podoviridae* phage PEV2 active against *Pseudomonas* using a Büchi B-290 small-scale spray dryer. Formulation with 80% trehalose and 20% leucine outperformed formulations containing different excipient ratios or mannitol in terms of titer reduction after one year of refrigerated storage at 0% or 22% relative humidity (Leung et al., 2017). They also showed that spray-dried phage PEV2 and *Myoviridae* phage PEV40 formulated using leucine and trehalose were stable for one year at refrigerated or 20°C storage using vacuum packaging (Leung et al., 2018). Chang et al. (2017) demonstrated that, out of many different excipients (trehalose, lactose, mannitol, glycine, leucine, PEG3000, and pluronic F-68) and mass fractions tested with *Podoviridae* phage PEV1, the leading formulation contained 17 mg/mL trehalose and 8 mg/mL leucine, for which only $0.2 \log_{10}(\text{PFU/mL})$ titer reduction was observed. However, other formulations provided better stabilization to other phages, and this combined with the differences in titer

reduction in the aforementioned studies, indicates that the manufacturability of phages varies such that it needs assessment for each phage.

The feasibility of spray drying anti-*Campylobacter* phages has not previously been assessed.

In this paper, a spray drying process is developed for producing flowable anti-

95 *Campylobacter* phage powder using leucine and trehalose excipients that retains biological activity after room temperature storage and long distance shipping in suitable packaging.

2 Materials and Methods

2.1 Phage Stocks

Phage CP30A (vB_CjeM_CP30A; NCBI nucleotide accession JX569801) is group III phage
100 (Fletchervirus) with characteristic genome sizes of approximately 130-135 kb; CP20
(vB_CcoM_CP20; NCBI nucleotide accession MK408758) is a group II phage
(Firehammervirus) with a characteristic genome sizes of approximately 175-183 kb (Javed et al., 2014). Both are *Eucampyvirinae* of the family *Myoviridae* that infect a range of *C. jejuni* or *C. coli* host bacteria. These phages were isolated from chicken excreta and are present in
105 chicken gut (Loc Carrillo et al., 2005; Scott et al., 2007a; Scott et al., 2007b; Siringan et al., 2011; Siringan et al., 2014; Javed et al., 2014; Brathwaite et al., 2015; Richards et al., 2019).
A transmission electron micrograph (TEM) of phage CP30A is given in Figure 1.

[Insert Figure 1]

2.2 Phage Amplification

110 High titer phage lysates were generated by adding 100 µl of phages at 7 log₁₀(PFU/mL) to 500 µL of bacterial suspension at 8 log₁₀(CFU/mL). This mixture was then transferred to 5 mL of melted NZCYM overlay agar, which was pre-tempered to 50°C, and poured onto NZCYM

agar plate. After overnight incubation at 42°C under microaerobic conditions, the plates were then flooded with water. The host strain was isolated from United Kingdom broiler flocks in a previous study (Loc Carrillo et al., 2005). The propagation process is discussed in detail elsewhere (Cairns et al., 2009). The amplified lysate was passed through a 0.22 µm filter. The filtered lysate had a solids content of ~19 mg/mL, as measured post-evaporation of suspensions on pre-weighed microscope slides. The filtered lysate was optionally further purified 30 mL at a time by centrifugation at $3.4 \times 10^5 \text{ m/s}^2$ [$35,000 \times g$] for 120 minutes at 4°C. After centrifugation, the supernatant was drained and the pellet was resuspended in 30 mL of reverse osmosis water. The measured solids content of the pellet suspension was then less than 0.5 mg/mL. The titer of lysates after filtration or filtration and centrifugation was approximately 9.5 log(PFU/mL).

2.3 Formulation

The formulation excipients were L-leucine (Code 125121000, lot A0269620; Acrōs Organics, NJ, USA) and D-(+)-trehalose dihydrate (Cat. no. BP2687; Fisher BioReagents, NH, USA). The dissolved excipient concentrations were 7.5 mg/mL leucine and 22.5 mg/mL trehalose for most experiments, similar to the leading formulation for spray drying phages in a previous study (Chang et al., 2017). Higher dissolved excipient concentrations, 20 mg/mL leucine and 100 mg/mL trehalose, were also tested and used for shipping experiments. Comparisons were made between adding the excipients directly to the filtered lysate and diluting the filtered and centrifuged lysate 1:100 into pre-prepared excipient solution.

2.4 Air Drying

To determine whether desiccation is a major cause of titer reduction, filtered phage lysates were air-dried at room temperature for 48 hours in a Petri dish (92 mm diameter; Sarstedt,

Leicestershire, UK) and assayed. TEM was performed on filtered and centrifuged phage CP20 lysate that was air-dried on a TEM mesh stub to examine potential mechanisms of inactivation due to desiccation.

2.5 Atomization

140 A titer reduction due to atomization comparison was made between the use of a vibrating mesh nebulizer (Aerogen Solo; Model no. 06675745, Lot 60201509300103, Ref AG-AS3350-US; Aerogen Ltd., Dangan, Galway, Ireland) with Pro-X Controller (S/N AP-1510412, Ref AG-PX-1050-IN; Aerogen Ltd., Dangan, Galway, Ireland) and a custom twin-fluid atomizer. For these measurements, 3 mL of filtered lysate with direct addition of leucine and trehalose
145 was atomized directly onto a filter (Respirgard II bacterial/viral filter, ref 303EU, lot 12127233; Vital Signs, Inc., Englewood, CO, USA), as per Figure 2. A custom adapter made from rigid opaque material simulating plastic (Objet VeroGray RGD850; Stratsys, Ltd., Eden Prairie, MN, USA), using a PolyJet 3D printer (Objet Eden 350V High Resolution 3D Printer; Stratsys, Ltd., Eden Prairie, MN, USA) connected the filter to the twin-fluid atomizer. No
150 adapter was used for the vibrating mesh nebulizer as it fit into the filter. The atomized liquid remained suspended on the filter fibers and 10 mL of buffer was added and mixed by swirling the filter. The mixture was drawn from the filter space using a micropipette and assayed.

[Insert Figure 2]

155 The vibrating mesh nebulizer generates droplets of an initial diameter of approximately 5.5 μm [Martin et al., 2011]. For the twin-fluid atomizer, an atomizing gas (nitrogen) flow rate of 1.5×10^{-4} kg/s and a liquid spray rate of 1.7×10^{-5} kg/s were used. The initial mass median droplet diameter was approximately 9 μm for these air-liquid ratio settings according to

data presented elsewhere (Hoe et al., 2014b). At this atomizing gas flow rate and spray rate
160 setting about 13% of the droplet volume evaporates to fully humidify the atomizing gas. As
there was no spray drying gas for these measurements, this results in a relatively small
amount of droplet desiccation, thus minimizing contact of the phages with the air-liquid
interface, and ensuring the observed titer reduction is due primarily to shear stress. A
characteristic shear rate on the order of $1 \times 10^5 \text{ s}^{-1}$ was expected for the twin-fluid atomizer
165 according to a model presented by Ghandi et al. (2012). Phages have remained active after
atomization using a similar shear rate (Leung et al., 2016).

2.6 Spray Drying

A comparison of overall titer reduction due to spray drying was made between a modified
Büchi B-90 spray dryer (Büchi Labortechnik AG; Flawil, Switzerland) that uses the vibrating
170 mesh nebulizer and a Büchi B-191 spray dryer (Büchi Labortechnik AG, Flawil, Switzerland)
with upgraded process analytics and controls that uses the custom twin-fluid atomizer.

Process schematics of the spray dryer installations are given in Figure 3. In the modified
Büchi B-90 design, the nebulizer was placed outside the spray drying chamber to minimize
heat exposure of the phages prior to atomization. The collected powder was scraped from
175 the collecting electrode onto collection paper and then transferred into a tube for assay. In
the Büchi B-191 design the formulation was fed to the custom twin-fluid atomizer using a
peristaltic pump (Model no. 7528-30, Masterflex L/S, with pump head model no. 77200-60,
Easy-load II; Cole-Parmer, Vernon Hills, IL, USA). The powder was captured in a collection
bottle using a cyclone.

180 For both spray dryers an inlet temperature of 70°C was used, which is similar to values that,
in the literature (Matinkhoo et al., 2011; Leung et al., 2016; Leung et al., 2017; Leung et al.,

2018), worked well for spray drying phages. The surface temperature of the atomized droplets is typically assumed to remain near the wet bulb temperature due to evaporative cooling (Masters, 1972); therefore, thermal inactivation of the phages is not expected during initial stages of solvent evaporation. To demonstrate this, additional measurements with the Büchi B-191 spray dryer were performed using an inlet temperature of 50°C and the level of phage inactivation compared to use of an inlet temperature of 70°C. The drying gas flow rate was 100 L/min for the B-90 and 425 L/min for the B-191. The spray rate was measured to be 0.1 mL/min for the B-90 and set and measured to be 1 mL/min for the B-191. Using a process model described elsewhere (Carrigy & Vehring, 2019), for an inlet temperature of 70°C, the predicted outlet temperature and outlet relative humidity values were 44°C and 1.6% for the B-90 and 49°C and 2.9% relative humidity for the B-191. The moisture content in the powder, neglecting the small effect of leucine on moisture uptake, was thus expected to be ~0.4% for the B-90 and ~0.7% for the B-191 based on trehalose moisture uptake data from the literature (Carrigy & Vehring, 2019). Both of these moisture contents are suitable for long-term physical stability (retention of amorphous structure) of trehalose for dry room temperature storage conditions, according to a trehalose-water supplemented phase diagram (Carrigy & Vehring, 2019). This is important since the trehalose must remain amorphous to ensure glass stabilization; crystallization of trehalose has been shown to inactivate phages (Vandenheuvel et al., 2014). Overall titer reduction (filtered lysate titer, divided by 100 if diluted, minus equivalent powder titer) comparison was made between formulations using leucine and trehalose, and 'Neat' filtered lysates not containing any additional excipients as controls to determine if the excipients were improving the titer reduction or not.

205 Scanning electron microscope (SEM) images of spray-dried powder were taken using a field-emission SEM (Zeiss Sigma FESEM, Oberkochen, Germany) at 5000x magnification, using an immersion lens (in-lens) detector, a working distance of ~7 mm, and an accelerating voltage of 2 kV. A gold coating of ~10 nm was applied prior to imaging with a sputter deposition system to minimize charging (Denton II; Denton Vacuum LLC, Moorestown, NJ, USA).

210 [Insert Figure 3]

2.7 Powder Packaging and Shipping

Powder packaging was performed by transferring the collected powder into 5 mL tubes (Catalogue no. 0030119487, lot G1710906; Eppendorf AG, Hamburg, Germany) that were double heat-sealed in double-bagged aluminium foil bags (Prod no. 139-313; Ted Pella, Inc., Redding, CA, USA) with molecular sieve desiccant packs (Cat no. 1523T76; McMaster Carr, Aurora, OH, USA), as per Figure 4. Heat sealing was performed using an impulse sealer (No. 912951, type AIE-300; American International Electric, Inc., City of Industry, CA, USA). The packaging components were first placed in a nitrogen-purged enclosure set to 25°C and approximately 0% relative humidity for 4-10 hours to remove moisture. After spray drying with the Büchi B-191, the collection bottle (see Figure 3) was removed, quickly capped, and placed in the nitrogen-purged enclosure, where the powder was added to the already dried out tube. Packaging and heat sealing then took place in the nitrogen-purged enclosure. The solid phase of the spray-dried powder stored in the packaging at room temperature for 4 months was determined using a custom Raman spectrometer developed by Wang et al. (2014).

225 [Insert Figure 4]

Packages were shipped between Edmonton, Canada and Nottingham, England (~6,600 km one-way) under ambient temperature storage conditions. The shipment contained aluminium foil packages surrounded by packing paper and placed in a Styrofoam box within a cardboard box. The temperature in the boxes during shipment was recorded with a temperature monitor (TempTale 4 USB MultiAlarm; Sensitech Canada, Markham, ON, Canada). Upon arrival, the dry packages were stored at room temperature in the laboratory until just prior to assay, at which time they were opened.

The timeline and protocol of the shipping study is shown in Figure 5. The same large batch of spray-dried phage powder was aliquoted into 4 tubes which were each packaged into separate aluminium foil packages, termed LT(1), LT(2), LT(3), and LT(4). Package LT(1) was shipped and assayed soon after spray drying and is considered the control measurement as all spray drying occurred in Canada and all assays in England. Package LT(2) was shipped soon after spray drying but was not assayed for 3 weeks. Package LT(3) was shipped to England soon after packaging, then shipped back to Canada, then shipped back to England (~19,800 km total), and then assayed at the same time as LT(2) and LT(4). Package LT(4) was kept in Canada and sent in a later shipment to England for assay. Comparison of LT(1) and LT(2) titer reduction indicated whether 3 weeks of room temperature storage caused phage inactivation. Comparison of LT(3) to the other packages indicated whether multiple shipments caused additional titer reduction (e.g. due to shaking during shipping and handling). Comparison of LT(1) and LT(4) titer reduction indicated whether different shipments caused different phage titer reductions.

[Insert Figure 5]

2.8 Plaque Assays

Plaque assays were performed using *C. jejuni* as the host strain to determine biological activity of the phages. Spot assays were performed in triplicate at multiple dilution levels. For powder, it was necessary to redissolve into water at a concentration of 30 mg/mL prior to performing plaque assay to determine titer. No clear trend in recovered titer was observed using buffer or water at different temperatures (4°C, 20°C, 37°C) to redissolve the powder.

2.9 Statistics

Results are generally presented as mean \pm standard deviation. Titer results are based on spot assays performed in triplicate. The standard deviation in titer reduction was obtained by error propagation for subtraction, with the individual standard deviations representing the errors. Statistical comparisons utilized the Student's t-test without assuming equal variance, at a significance level of 0.05.

3 Results and Discussion

3.1 Air Drying

The titer reductions due to air drying in a Petri dish were $2.0 \pm 0.1 \log_{10}(\text{PFU/mL})$ for filtered 'Neat' CP30A lysate, $1.8 \pm 0.3 \log_{10}(\text{PFU/mL})$ for filtered CP30A lysate with direct addition of 7.5 mg/mL leucine and 22.5 mg/mL trehalose, $2.0 \pm 0.2 \log_{10}(\text{PFU/mL})$ for filtered and centrifuged CP30A lysate with direct addition of 7.5 mg/mL leucine and 22.5 mg/mL trehalose, and $3.5 \pm 0.2 \log_{10}(\text{PFU/mL})$ for filtered and centrifuged CP30A lysate. As the filtered and centrifuged lysate without leucine and trehalose had significantly greater titer reduction than the other cases ($p < 0.001$), it was apparent that leucine or trehalose, and impurities in the lysate, both provide some biological stabilization against desiccation.

3.2 Atomization

The titer reduction upon atomization with the vibrating mesh nebulizer was 0.8 ± 0.2

$\log_{10}(\text{PFU/mL})$ while the titer reduction upon atomization with the twin-fluid atomizer was

275 $0.4 \pm 0.2 \log_{10}(\text{PFU/mL})$, which was significantly less ($p < 0.05$). Shear stress during twin-fluid

atomization was therefore not a major cause of titer reduction for phage CP30A. The results

indicate that the choice of atomization method is important for maximizing biological

activity. The twin-fluid atomization titer reduction was less than the value of ~ 0.75

$\log_{10}(\text{PFU/mL})$ reported in the literature (Leung et al., 2016) for anti-*Pseudomonas* phage

280 PEV2 (*Podoviridae*). Similar shear rates occurred in that study, but greater droplet diameter

reduction via evaporation and hence greater desiccation stress may have occurred. The

smaller titer reduction observed in this study may also be due to differences in susceptibility

between phage families. The vibrating mesh nebulizer titer reduction was greater than the

value of approximately $0.4 \log_{10}(\text{PFU/mL})$ for anti-tuberculosis phage D29 (*Siphoviridae*) in

285 isotonic saline calculated from the literature (Carrigy et al., 2017), indicating that phage

CP30A is potentially more susceptible to titer reduction with this device than phage D29.

This again may be due to differences in susceptibility between phage families.

3.3 Spray Drying

The outlet temperatures matched predictions from the process models to within 2°C for

290 both spray dryers. A comparison of spray drying filtered phage CP30A lysate with direct

addition of 7.5 mg/mL leucine and 22.5 mg/mL trehalose using different spray dryers is

given in Table 1. Use of the B-191 spray dryer resulted in significantly less ($p < 0.025$) overall

titer reduction than use of the B-90 spray dryer, thus providing some evidence that attrition

during cyclone collection with the B-191 was not a major cause of titer reduction, as the B-

90 spray dryer does not have a cyclone. Non-significant differences ($p > 0.5$) in titer reduction between the spray dryers occurred when the effects of atomization (results in Section 3.2) were subtracted from the overall titer reductions, indicating that another common factor, likely desiccation, was a more substantial cause of titer reduction. Since the B-191 resulted in less overall titer reduction, it was used for all further spray drying experiments. The titer of the powder for the B-191 was approximately 4×10^8 PFU/g. This is a high titer and demonstrates the feasibility of production of phage CP30A dry powder via spray drying. Whether this titer is suitable for *Campylobacter* decontamination and biocontrol remains a subject of future research.

[Insert Table 1]

The results of titer reduction due to spray drying excluding atomization were not significantly different ($p > 0.2$) from the titer reduction for the corresponding air drying case from Section 3.1, $1.8 \pm 0.3 \log_{10}(\text{PFU/mL})$, providing further evidence that desiccation stress was the main cause of titer reduction. This was not unexpected, as literature indicates that many phages are susceptible to desiccation stress (Cambell-Renton, 1941). Larger overall titer reductions were observed as compared to other phages in the literature using similar formulation (see Introduction), indicating these anti-*Campylobacter* phages may be highly susceptible to inactivation by desiccation. This provides evidence that phage formulation and processing has to be developed on a case-by-case basis.

The overall titer reduction for filtered and centrifuged phage lysates (CP30A and CP20) diluted into leucine and trehalose and spray-dried using the Büchi B-191 with inlet temperatures of either 70°C or 50°C are given in Table 2. None of the overall titer reduction results were significantly different ($p > 0.1$) indicating that heat stress due to these

moderate drying gas inlet temperatures was not a likely cause of titer reduction and that the anti-*Campylobacter* phages tested had similar susceptibility. The overall titer reductions

320 due to spray drying the 'Neat' filtered lysates at 70°C were $2.5 \pm 0.1 \log_{10}(\text{PFU/mL})$ for CP30A and $2.8 \pm 0.2 \log_{10}(\text{PFU/mL})$ for CP20. Excluding atomization, the titer reduction for spray drying 'Neat' phage CP30A was not significantly different ($p > 0.2$) than the corresponding air drying case, in agreement with the previous results suggesting that desiccation was the main cause of titer reduction. Spray drying 'Neat' filtered lysates
325 resulted in significantly greater ($p < 0.01$) titer reductions than spray drying with leucine and trehalose for both phage types at the same inlet temperature of 70°C. This indicated that use of leucine and trehalose improved biological stability relative to the case without excipients. TEM images of desiccated phage CP20 are given in Figure 6. It was observed that many desiccated phages had burst capsids and leaked DNA that would render them inactive.
330 TEM (not shown) also indicated that some phages without burst capsids appeared damaged or had contracted tails that would render them inactive.

[Insert Figure 6]

[Insert Table 2]

The SEM images in Figure 7 demonstrate that with centrifugation and dilution of the filtered
335 phage lysate, the spray-dried particle morphology consisted of a semi-spherical shape with some dimples. This was comparable to the spray-dried leucine and trehalose vehicle, but was not as collapsed. On the other hand, the spray-dried 'Neat' filtered lysate particles were wrinkled and similar to spray-dried filtered lysate particles generated without centrifugation and dilution steps. This indicated that the impurities concentrated on the surface and

340 affected the particle morphology and the lowering of impurity concentration by centrifugation and dilution minimizes this surface concentration effect.

[Insert Figure 7]

Using TEM (Figure 8), it was observed that the impurities were mainly bacterial debris that had passed through the 0.22 μm filter. This debris may contain a number of bacterial
345 components including amino acids, lipids, and sugars, among others.

[Insert Figure 8]

The surface concentration of the phages, excipients, and impurities is controlled by the Péclet number, a dimensionless number used to characterize the ratio of surface recession rate due to droplet evaporation and diffusional rate of the solute and suspended matter to
350 the interior of the droplet (Vehring, 2008). A high Péclet number (> 1) indicates the solute and suspended matter will concentrate on the surface, while a low Péclet number (< 1) indicates the solute and suspended matter will be relatively evenly distributed (Vehring, 2008). Furthermore, the surface enrichment is defined as the ratio of surface concentration of a component to its mean bulk concentration; surface enrichment increases with
355 increasing Péclet number (Boraey & Vehring, 2014). The reason the impurities affected the surface morphology is probably that at least some of the impurity components are large and slowly diffusing, thus having a high Péclet number, surface enrichment, and surface concentration, which affected particle formation and shell deformation. The phages are also relatively large and therefore have a high Péclet number and surface enrichment. Thus, it is
360 possible that the phages would tend to come into contact with the impurities at the surface, which could be detrimental as the impurities are unlikely to be effective glass stabilizers and may physically separate the phages from the stabilizing trehalose. Therefore, the

effectiveness of excipients likely depends on the level of purification and the phage preparation method.

365 Relative to the overall titer reduction results in Table 1, the results in Table 2 indicate that the use of centrifugation and dilution of the filtered lysate resulted in significantly less ($p < 0.025$), $\sim 0.5 \log_{10}(\text{PFU/mL})$ less, overall titer reduction due to spray drying, than adding the excipients directly to the filtered phage lysate. This is likely because centrifugation and dilution resulted in a mean impurity concentration in the formulation of less than 0.005
370 mg/mL, whereas without it the filtered lysate had a mean impurity concentration of ~ 19 mg/mL. Even with high surface enrichment, the surface concentration of impurities if centrifugation and dilution steps are used is relatively small. Considering also the higher overall titer reduction upon spray drying 'Neat' filtered lysate rather than that with leucine and trehalose, it was deduced that the impurities in the filtered phage lysate did not provide
375 effective biological stabilization and should be minimized in phage formulation for spray drying.

In view of the small batch size of 600 mg, good manufacturability was observed with a high spray drying yield (mass collected in the collection bottle relative to the total formulated mass of solute and suspended matter) of 72-80%, for centrifuged and diluted filtered phage
380 lysates spray-dried with leucine and trehalose. This was much higher than the yield for the 'Neat' powders of 5-26%, indicating the leucine and trehalose substantially decreased the cohesiveness. The leucine and trehalose addition generated a white powder with improved flowability and allowed for easier powder handling and filling into vials with no noticeable electrostatic problems.

385 *3.4 Shipping Stability*

The temperature during shipment of packages LT(1), LT(2), and LT(3) from Canada to England was $23 \pm 7^{\circ}\text{C}$ (measured every 4 minutes), with a maximum excursion of 38°C for approximately 1.5 hours; the temperature remained above 35°C for approximately 4.5 hours. The temperature during shipment of LT(3) from England to Canada was $22 \pm 2^{\circ}\text{C}$, with
390 a maximum temperature of 24°C . The temperature during shipment of LT(3) and LT(4) from Canada to England was $22 \pm 4^{\circ}\text{C}$, with a maximum excursion of 29°C for approximately 1.5 hours.

De-convoluted Raman spectra of the powder indicated that the leucine was partially crystalline. The trehalose was fully amorphous, as expected (see Section 2.6), and therefore
395 capable of preserving biological activity by glass stabilization.

The overall titer reduction for LT(1) was $1.9 \pm 0.1 \log_{10}(\text{PFU/mL})$, which was not significantly different ($p > 0.2$) than the titer reduction of $2.0 \pm 0.2 \log_{10}(\text{PFU/mL})$ for the corresponding air drying case without shipping. This indicated that the titer reduction was most likely caused by desiccation during processing in all cases and not by shipping. This was
400 substantiated by shipping multiple times, with additional titer reductions for LT(2), LT(3), and LT(4) of $0.1 \pm 0.2 \log_{10}(\text{PFU/mL})$, $0.0 \pm 0.1 \log_{10}(\text{PFU/mL})$, and $0.1 \pm 0.1 \log_{10}(\text{PFU/mL})$, respectively. There were no significant differences in titer reduction between any of these powders ($p > 0.05$), indicating that shipping the packaged powder three times instead of once was not a cause of additional titer reduction and that 3 weeks of room temperature
405 storage in the packaging was not a cause of additional titer reduction. To our knowledge, this is the first shipping stability study that demonstrates that the cold chain is not necessary with proper formulation and packaging of phage powder.

4 Conclusions

Biologically-active and thermally-stable dry powder containing anti-*Campylobacter* phage

410 can be produced by spray drying. Maximizing biological activity requires a prudent choice of atomization method. Use of a twin-fluid atomizer resulted in negligible harm to the phages due to shear stress and is recommended over use of a vibrating mesh nebulizer. The effectiveness of excipients at preserving biological activity depends on the level of purification and the phage preparation method. Centrifuging and diluting the filtered phage
415 lysate into leucine and trehalose prior to spray drying improved biological activity retention relative to use of no excipients or incorporation of excipients directly into the filtered lysate, due to minimization of lysate impurity concentrations. The generated low cost powder was flowable and non-cohesive, and exhibited a high manufacturing yield. The developed packaging method for controlling moisture level and hence maintaining a sufficiently high
420 glass transition temperature to prevent trehalose crystallization may be useful in many global health applications requiring global distribution without cold chain infrastructure. This was demonstrated by successful retention of biological activity following 3 weeks of room temperature storage and long distance ambient temperature shipping with measured temperature excursion. Although use of leucine and trehalose improved biological stability
425 relative to use of no excipients, future work is of interest to further decrease biological activity losses due to desiccation, for example, by incorporation of different excipients. As phages have different susceptibilities to desiccation stress, formulation efforts should be phage-specific and begin early in the pharmaceutical development process.

Acknowledgements

430 NC thanks the Killam Trusts, the Natural Sciences and Engineering Research Council of
Canada, Alberta Innovates, and the University of Alberta for scholarship funding. NC thanks
Arlene Oatway for help with TEM.

Funding

This work was financially supported by the United Kingdom Research and Innovation,
435 Biotechnology and Biological Sciences Research Council [grant number BB/P02355X/1]. The
funding source had no role in study design, collection, analysis, or interpretation of data,
writing the article, or in decision to submit the article for publication.

References

- Anandharamakrishnan, C., Padma Ishwarya, S., 2015. Spray drying techniques for food
440 ingredient encapsulation. John Wiley & Sons, Ltd., Chichester.
<https://doi.org/10.1002/9781118863985>.
- Boraey, M.A., Vehring, R., 2014. Diffusion controlled formation of microparticles. J. Aerosol
Sci. 67, 131-143. <https://doi.org/10.1016/j.jaerosci.2013.10.002>.
- Brathwaite, K. J., Siringan, P., Connerton, P. L., Connerton, I. F., 2015. Host adaption to the
445 bacteriophage carrier state of *Campylobacter jejuni*. Res. Microbiol. 166, 504–515.
<https://doi.org/10.1016/j.resmic.2015.05.003>.
- Cairns, B.J., Timms, A.R., Jansen, V.A.A., Connerton, I.F., Payne, R.J.H., 2009. Quantitative
models of *in vitro* bacteriophage-host dynamics and their application to phage therapy. PLoS
Pathog. 5, e1000253. <https://doi.org/10.1371/journal.ppat.1000253>.
- 450 Cambell-Renton, M.L., 1941. Experiments on drying and on freezing bacteriophage. J.
Pathol. 53, 371-384. <https://doi.org/10.1002/path.1700530304>.

- Carrigy, N.B., Chang, R.Y., Leung, S.S.Y., Harrison, M., Petrova, Z., Pope, W.H., Hatfull, G.F., Britton, W.J., Chan, H.K., Sauvageau, D., Finlay, W.H., Vehring, R., 2017. Anti-tuberculosis bacteriophage D29 delivery with a vibrating mesh nebulizer, jet nebulizer, and soft mist inhaler. *Pharm. Res.* 34, 2084-2096. <https://doi.org/10.1007/s11095-017-2213-4>.
- Carrigy, N.B., Vehring R., 2019. Engineering stable spray-dried biologic powder for inhalation. In: Hickey, A.J., da Rocha, S.R.P. (Eds.), *Pharmaceutical Inhalation Aerosol Technology*, third ed. CRC Press, Boca Raton, pp. 291-326. <https://doi.org/10.1201/9780429055201>.
- Carron, M., Chang, Y.-M., Momanyi, K., Akoko, J., Kiiru, J., Bettridge, J., Chaloner, G., Rushton, J., O'Brien, S., Williams, N., Fèvre, E.M., Häsler, B., 2018. *Campylobacter*, a zoonotic pathogen of global importance: prevalence and risk factors in the fast-evolving chicken meat system of Nairobi, Kenya. *PLoS Negl. Trop. Dis.* 12, e0006658. <https://doi.org/10.1371/journal.pntd.0006658>.
- Chang, R.Y., Wong, J., Mathai, A., Morales, S., Kutter, E., Britton, W., Li, J., Chan, H.K., 2017. Production of highly stable spray dried phage formulations for the treatment of *Pseudomonas aeruginosa* lung infection. *Eur. J. Pharm. Biopharm.* 121, 1-13. <https://doi.org/10.1016/j.ejpb.2017.09.002>.
- Desai, K.G.H., Park, H.J., 2005. Recent developments in microencapsulation of food ingredients. *Drying Technol.* 23, 1361-1394. <https://doi.org/10.1081/DRT-200063478>.
- Firleyanti, A.S., Connerton, P.L., Connerton, I.F., 2016. Campylobacters and their bacteriophages from chicken liver: the prospect for phage biocontrol. *Int. J. Food Microbiol.* 237, 121-127. <https://doi.org/10.1016/j.ijfoodmicro.2016.08.026>.
- Ghandi, A., Powell, I.B., Howes, T., Chen, X.D., Adhikari, B., 2012. Effect of shear rate and oxygen stresses on the survival of *Lactococcus lactis* during the atomization and drying

stages of spray drying: a laboratory and pilot scale study. J. Food Eng. 113, 194-200.

<https://doi.org/10.1016/j.jfoodeng.2012.06.005>.

Hoe, S., Boraey, M.A., Ivey, J.W., Finlay, W.H., Vehring, R., 2014. Manufacturing and device options for the delivery of biotherapeutics. J. Aerosol Med. Pulm. Drug Deliv. 27, 315-328.

480 <https://doi.org/10.1089/jamp.2013.1090>.

Hoe, S., Ivey, J.W., Boraey, M.A., Shamsaddini-Shahrbabak, A., Javaheri, E., Matinkhoo, S., Finlay, W.H., Vehring, R., 2014b. Use of a fundamental approach to spray-drying formulation design to facilitate the development of multi-component dry powder aerosols for respiratory drug delivery. Pharm. Res. 31, 449-465.

485 Holsinger, V.H., McAloon, A.J., Onwulata, C.I., Smith, P.W., 2000. A cost analysis of encapsulated spray-dried milk fat. J. Dairy Sci. 83, 2361-2365.

[https://doi.org/10.3168/jds.S0022-0302\(00\)75124-1](https://doi.org/10.3168/jds.S0022-0302(00)75124-1).

Huang, S., Vignolles, M.-L., Chen, X.D., Le Loir, Y., Jan, G., Schuck, P., Jeantet, R., 2017. Spray drying of probiotics and other food-grade bacteria: a review. Trends Food Sci. Technol. 63,

490 1-17. <https://doi.org/10.1016/j.tifs.2017.02.007>.

Janež, N., Kokošin, A., Zaletel, E., Vranac, T., Kovač, J., Vučković, D., Smole Možina, S., Curin Šerbec, V., Zhang, Q., Accetto, T., Podgornik, A., Peterka, M., 2014. Identification and characterization of new *Campylobacter* group III phages of animal origin. FEMS Microbiol. Lett. 359, 64-71. <https://doi.org/10.1111/1574-6968.12556>.

495 Javed, M.A., Ackermann, M.-W., Azeredo, J., Carvalho, C.M., Connerton, I., Evoy, S., Hammerl, J.A., Hertwig, S., Lavigne, R., Singh, A., Szymanski, C., Timms, A., Kropinski, A.M., 2014. A suggested classification for two groups of *Campylobacter* myoviruses. Arch. Virol. 159, 181-190. <https://doi.org/10.1007/s00705-013-1788-2>.

Kaakoush, N.O., Castaño-Rodríguez, N., Mitchell, H.M., Man, S.M., 2015. Global
 500 epidemiology of *Campylobacter* infection. Clin. Microbiol. Rev. 28, 687-720.
<https://doi.org/10.1128/CMR.00006-15>.

Kittler, S., Fischer, S., Abdulmawjood, A., Glünder, G., Klein, G., 2013. Effect of
 bacteriophage application on *Campylobacter jejuni* loads in commercial broiler flocks. Appl.
 Env. Microbiol. 79, 7525-7533. <https://doi.org/10.1128/AEM.02703-13>.

505 Kutter, E., Sulakvelidze, A., 2005. Bacteriophages: Biology and Applications. CRC Press, Boca
 Raton.

Kutter, E., de Vos, D., Gvasalia, G., Alavidze, Z., Gogokhia, L., Kuhl, S., Abedon, S.T., 2010.
 Phage therapy in clinical practice: treatment of human infections. Curr. Pharm. Biotechnol.
 11, 69-86. <https://doi.org/10.2174/138920110790725401>.

510 Ledet, G.A., Graves, R.A., Bostanian, L.A., Mandal, T.K., 2015. Spray-drying of
 biopharmaceuticals. In: Varshney, D., Singh, M. (Eds.), Lyophilized Biologics and Vaccines:
 Modality-based Approaches. Springer Science+Business Media, New York, pp. 273-297.
https://doi.org/10.1007/978-1-4939-2383-0_12.

Leung, S.S.Y., Parumasivam, T., Gao, F.G., Carrigy, N.B., Vehring, R., Finlay, W.H., Morales, S.,
 515 Britton, W.J., Kutter, E., Chan, H.K., 2016. Production of inhalation phage powders using
 spray freeze drying and spray drying techniques for treatment of respiratory infections.
 Pharm. Res. 33, 1486-1496. <https://doi.org/10.1007/s11095-016-1892-6>.

Leung, S.S.Y., Parumasivam, T., Gao, F.G., Carter, E.A., Carrigy, N.B., Vehring, R., Finlay, W.H.,
 Morales, S., Britton, W.J., Kutter, E., Chan, H.K., 2017. Effect of storage conditions on the
 520 stability of spray dried, inhalable bacteriophage powders. Int. J. Pharm. 521, 141-149.
<https://doi.org/10.1016/j.ijpharm.2017.01.060>.

- Leung, S.S.Y., Parumasivam, T., Nguyen, A., Gengenbach, T., Carter, E.A., Carrigy, N.B., Wang, H., Vehring, R., Finlay, W.H., Morales, S., Britton, W.J., Kutter, E., Chan, H.K., 2018. Effect of storage temperature on the stability of spray dried bacteriophage powders. *Eur. J. Pharm. Biopharm.* 127, 213-222. <https://doi.org/10.1016/j.ejpb.2018.02.033>.
- 525 Loc Carrillo, C., Atterbury, R.J., El-Shibiny, A., Connerton, P.L., Dillon, E., Scott, A., Connerton, I.F., 2005. Bacteriophage therapy to reduce *Campylobacter jejuni* colonization of broiler chickens. *Appl. Env. Microbiol.* 71, 6554-6563. <https://doi.org/10.1128/AEM.71.11.6554-6563.2005>.
- 530 Loc-Carrillo, C., Abedon, S.T., 2011. Pros and cons of phage therapy. *Bacteriophage* 1, 111-114. <https://doi.org/10.4161/bact.1.2.14590>.
- Martin, A.R., Ang, A., Katz, I.M., Häussermann, S., Caillibotte, G., Texereau, J., 2011. An *in vitro* assessment of aerosol delivery through patient breathing circuits used with medical air or a helium-oxygen mixture. *J. Aerosol Med. Pulm. Drug Deliv.* 5, 225-234.
- 535 Masters, K., 1972. *Spray Drying: An Introduction to Principles, Operational Practice and Applications*. Leonard Hill, London.
- Matinkhoo, S., Lynch, K.H., Dennis, J.J., Finlay, W.H., Vehring, R., 2011. Spray-dried respirable powders containing bacteriophages for the treatment of pulmonary infections. *J. Pharm. Sci.* 100, 5197-5205. <https://doi.org/10.1002/jps.22715>.
- 540 Moore, J.E., Barton, M.D., Blair, I.S., Corcoran, D., Dooley, J.S.G., Fanning, S., Kempf, I., Lastovica, A.J., Lowery, C.J., Matsuda, M., McDowell, D.A., McMahon, A., Millar, B.C., Rao, J.R., Rooney, P.J., Seal, B.S., Snelling, W.J., Tolba, O., 2006. The epidemiology of antibiotic resistance in *Campylobacter*. *Microbes Infect.* 8, 1955-1966. <https://doi.org/10.1016/j.micinf.2005.12.030>.

- 545 O'Reilly, C.E., Jaron, P., Ochieng, B., Nyaguara, A., Tate, J.E., Parsons, M.B., Bopp, C.A., Williams, K.A., Vinjé, J., Blanton, E., Wannemuehler, K.A., Vulule, J., Laserson, K.F., Breiman, R.F., Feikin, D.R., Widdowson, M.A., Mintz, E., 2012. Risk factors for death among children less than 5 years old hospitalized with diarrhea in rural western Kenya, 2005-2007: a cohort study. *PLoS Med.* 9, e1001256. <https://doi.org/10.1371/journal.pmed.1001256>.
- 550 Osano, O., Arimi, S.M., 1999. Retail poultry and beef as sources of *Campylobacter jejuni*. *East Afr. Med. J.* 76, 141-143.
- Quinn, Jr., J.J., 1965. The economics of spray drying. *Ind. Eng. Chem.* 57, 35-37. <https://doi.org/10.1021/ie50661a006>.
- Richards, P.J., Connerton, P.L., Connerton, I.F., 2019. Phage biocontrol of *Campylobacter* 555 *jejuni* in chickens does not produce collateral effects on the gut microbiota. *Front. Microbiol.* 10, 476. <https://doi.org/10.3389/fmicb.2019.00476>.
- Roser, B., 1991. Trehalose, a new approach to premium dried foods. *Trends Food Sci. Technol.* 2, 166-169. [https://doi.org/10.1016/0924-2244\(91\)90671-5](https://doi.org/10.1016/0924-2244(91)90671-5).
- Schwartzbach, H., 2011. Achieving aseptic drying with spray drying technologies. 560 *Pharmaceut. Technol. Europe* 23, 90-92. <http://www.pharmtech.com/achieving-aseptic-drying-spray-drying-technologies>.
- Scott, A. E., Timms, A. R., Connerton, P. L., Carrillo, C. L., Radzum, K. A., Connerton, I. F., 2007a. Genome dynamics of *Campylobacter jejuni* in response to bacteriophage predation. *PLoS Pathog.* 3, e119. <https://doi.org/10.1371/journal.ppat.0030119>.
- 565 Scott, A. E., Timms, A. R., Connerton, P. L., El-Shibiny, A., Connerton, I. F., 2007b. Bacteriophage influence *Campylobacter jejuni* types populating broiler chickens. *Environ. Microbiol.* 9, 2341–2353. <https://doi.org/10.1111/j.1462-2920.2007.01351.x>.

- Siew, A., 2016. Exploring the use of aseptic spray drying in the manufacture of biopharmaceutical injectables. *Pharmaceut. Technol.* 40, 24-27.
- 570 <http://www.pharmtech.com/exploring-use-aseptic-spray-drying-manufacture-biopharmaceutical-injectables>.
- Siringan, P., Connerton, P. L., Payne, R. J., Connerton, I. F., 2011. Bacteriophage-mediated dispersal of *Campylobacter jejuni* biofilms. *Appl. Environ. Microbiol.* 77, 3320–3326.
- <https://doi.org/10.1128/AEM.02704-10>.
- 575 Siringan, P., Connerton, P. L., Cummings, N. J., Connerton, I. F., 2014. Alternative bacteriophage life cycles: the carrier state of *Campylobacter jejuni*. *Open Biol.* 4, 130200.
- <https://doi.org/10.1098/rsob.130200>.
- Vehring, R., 2008. Pharmaceutical particle engineering via spray drying. *Pharm. Res.* 25, 999-1022. <https://doi.org/10.1007/s11095-007-9475-1>.
- 580 Wagenaar, J.A., van Bergen, M.A.P., Mueller, M.A., Wassenaar, T.M., Carlton, R.M., 2005. Phage therapy reduces *Campylobacter jejuni* colonization in broilers. *Vet. Microbiol.* 109, 275-283. <https://doi.org/10.1016/j.vetmic.2005.06.002>.
- Wagenaar, J.A., French, N.P., Havelaar, A.H., 2013. Preventing *Campylobacter* at the source: why is it so difficult? *Clin. Infect. Dis.* 57, 1600-1606. <https://doi.org/10.1093/cid/cit555>.
- 585 Walters, R.H., Bhatnagar, B., Tchessalov, S., Izutsu, K.I., Tsumoto, K., Ohtake, S., 2014. Next generation drying technologies for pharmaceutical applications. *J. Pharm. Sci.* 103, 2673-2695. <https://doi.org/10.1002/jps.23998>.
- Wang, H., Boraey, M.A., Williams, L., Lechuga-Ballesteros, D., Vehring, R., 2014. Low-frequency shift dispersive Raman spectroscopy for the analysis of respirable dosage forms.
- 590 *Int. J. Pharm.* 469, 197-205. <https://doi.org/10.1016/j.ijpharm.2014.04.058>.

World Health Organization, Food and Agricultural Organization of the United Nations & World Organisation for Animal Health, 2012. The Global View of Campylobacteriosis: Report of an Expert Consultation, Utrecht, Netherlands, 9-11 July 2012.

<http://www.who.int/iris/handle/10665/80751>.

595 Vandenheuvel, D., Meeus, J., Lavigne, R., Van den Mooter, G., 2014. Instability of bacteriophages in spray-dried trehalose powders is caused by crystallization of the matrix. Int. J. Pharm. 472, 202-205. <https://doi.org/10.1016/j.ijpharm.2014.06.026>.

Vandenheuvel, D., Singh, A., Vandersteegen, K., Klumpp, J., Lavigne, R., Van den Mooter, G., 2013. Feasibility of spray drying bacteriophages into respirable powders to combat
600 pulmonary bacterial infections. Eur. J. Pharm. Biopharm. 84, 578-582.

<https://doi.org/10.1016/j.ejpb.2012.12.022>.

Figure Captions

605 **Figure 1.** TEM image of phage CP30A prepared using the method described by Carrigy et al. (2017).

Figure 2. Schematic of the experiments used to determine the titer reduction due to atomization for a vibrating mesh nebulizer (left) and a custom twin-fluid atomizer (right). Full resolution colour image available online.

610 **Figure 3.** Process schematics of the spray dryer installations for the modified Büchi B-90 (top) and the Büchi B-191 with upgraded process analytics and controls (bottom). Important variables are denoted.

Figure 4. Dry packaging method. Full resolution colour image available online.

Figure 5. Timeline and protocol of the shipment study. Measurements were performed
615 using different shipments containing powder, composed of filtered and centrifuged phage CP30A lysate diluted 1:100 in 20 mg/mL leucine and 100 mg/mL trehalose. This powder was from the same spray-dried batch aliquoted into different tubes, each shipped in different packages.

Figure 6. TEM images showing DNA leaks from burst capsids of desiccated (air dried) phage
620 CP20. A TEM image of phage CP20 without a burst capsid was shown in Figure 1.

Figure 7. SEM images of powder spray-dried using a 70°C inlet temperature. Top-left: spray-dried 'Neat' filtered CP30A lysate; titer reduction $2.5 \pm 0.1 \log_{10}(\text{PFU/mL})$. Top-right: spray-dried filtered CP30A lysate with direct addition of 7.5 mg/mL leucine and 22.5 g/mL trehalose; titer reduction $2.3 \pm 0.2 \log_{10}(\text{PFU/mL})$ as per Table 1. Bottom-left: spray-dried
625 filtered CP30A lysate centrifuged and diluted (CD) 1:100 in 7.5 mg/mL leucine and 22.5 g/mL trehalose; titer reduction $1.8 \pm 0.2 \log_{10}(\text{PFU/mL})$ as per Table 2. Bottom-right: spray-dried 7.5 mg/mL leucine and 22.5 g/mL trehalose vehicle containing no phages. The microparticles

appeared similar for a 50°C inlet temperature and for phage CP20. The same scale bar applies to all images.

630 **Figure 8.** TEM showing bacterial debris that passed through the 0.22 µm filter.